PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference NIHA-0183	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/US2004/035831	International filing date *(day/month/year)* 27.10.2004	Priority date *(day/month/year)* 05.11.2003
International Patent Classification (IPC) or national classification and IPC A61K39/385, A61K47/48, A61K31/70		
Applicant THE GOVERNMENT OF THE UNITED STATES OF AM... et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 8 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
30.08.2005	24.10.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Ulbrecht, M Telephone No. +49 89 2399-7710

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US2004/035831

IAP20 Rec'd PCT/PTO 04 MAY 2006

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-43	as originally filed
Claims, Numbers	
1-13, 15-41, 43-50, 52-76	received on 01.09.2005 with letter of 30.08.2005
Drawings, Sheets	
1/18-18/18	as originally filed

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 43-76 (with respect to IA)

because:

☒ the said international application, or the said claims Nos. 43-76 (with respect to IA) relate to the following subject matter which does not require an international preliminary examination (specify):

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US2004/035831

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-13, 15-41, 43-50, 52-76
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-13, 15-41, 43-50, 52-76
Industrial applicability (IA)	Yes:	Claims	1-13, 15-41
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (SEPARATE SHEET)

International application No.
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Re item III.

Claims 43-50 and 52-76 relate to subject-matter considered by this Authority to be covered by the provisions of R. 67.1(iv) PCT, namely to methods of treatment of the human or animal body by therapy. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Art. 34(4)(a)(I) PCT).

Re item V.

1. Reference is made to the following documents:
   D1: WO 02/32404 A (CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS; KIDDLE, SIMON, JOHN;) 25 April 2002 (2002-04-25)
   D2: BARCHI JOSEPH J JR ET AL: "Synthesis and properties of carbohydrate- and glycopeptide-bearing nanoparticles." ABSTRACTS OF PAPERS AMERICAN CHEMICAL SOCIETY, vol. 226, no. 1-2, 2003, page CARB 40 & 226TH ACS (AMERICAN CHEMICAL SOCIETY) NATIONAL MEETING; NEW YORK, NY, USA; SEPTEMBER 07-11, 2003 ISSN: 0065-7727
   D3: HAKOMORI S: "ABERRANT GLYCOSYLATION IN TUMORS AND TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS" ADVANCES IN CANCER RESEARCH, ACADEMIC PRESS, LONDON, GB, vol. 52, 1989, pages 257-331
   D4: GLINSKY VLADISLAV V ET AL: "The role of Thomsen-Friedenreich antigen in adhesion of human breast and prostate cancer cells to the endothelium" CANCER RESEARCH, vol. 61, no. 12, 15 June 2001 (2001-06-15), pages 4851-4857

2. *Novelty (Art. 33(2) PCT):*

   The subject-matter of claims 1-13, 15-41, 43-50 and 52-76 is novel as the combination of features suggested by these claims is not disclosed in the prior art.

3. *Inventive step (Art. 33(3) PCT):*

3.1 D1 (p. 1, l. 4-10; p. 7, l. 24 - p. 8, l. 14; p. 14, l. 15-28; claims 26-27; Fig. 1) and D2

(abstract) disclose methods of preparing antigen-nanoparticle conjugates wherein a plurality of tumour-associated carbohydrate antigens is attached to the surface of a nanoparticle. The subject-matter of claim 1 differs from either D1 or D2 in that it refers to specific tumour-associated carbohydrate antigens. These antigens are, however, well-known to the skilled person (cf. e.g. D3 and D4) and appear to be arbitrarily selected by the skilled person when putting the teaching of D1 or D2 into practice. As they do not bring about any surprising technical effect the subject-matter of claim 1 is considered not inventive.

3.2 As D1 (supra; claim 1) and D2 (supra) also disclose the said antigen-nanoparticle conjugates, the considerations under V. 3.1 also apply to claim 12.

3.3 D1 (supra) explicitly and D2 (supra) implicitly disclose a method of inhibiting metastasis using said antigen-nanoparticle conjugates or of inhibiting the tumour cell binding to lectin-bearing endothelial cells ie metastasis. Hence, for the foregoing considerations (cf. V. 3.1) claim 43 is not considered inventive either.

3.4 For similar considerations as under V. 3.2 and 3.3 the subject-matter of claims 16, 17, 52 and 53 is not considered inventive either.

3.5 The additional features suggested by claims 2-5, 13, 15, 20, 21, 24-29, 32, 33, 36-40, 54, 60-64, 67, 68, and 72-76 are also explicitly or implicitly disclosed by D1 (supra; claims 2-4, 11, 15; p. 3, l. 22 -p. 8, l. 28) and thus do not establish an inventive step.

3.6 The additional features suggested by claims 6-11, 18, 19, 22, 23, 30, 31, 34, 35, 41, 44-50, 55-59, 65, 66 and 69-71 refer to routine modifications of the antigen-nanoparticles, of the method of preparing the said or of using them according to D1. These modifications are arbitrary and as not resulting in any surprising technical effect they do not establish an inventive step.

4. *Industrial applicability (Art. 33(4) PCT):*

4.1 For the assessment of the present claims 43-50 and 52-76 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States.

The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

4.2 Industrial applicability of the subject-matter of claims 1-13 and 15-41 is acknowledged.

5. *Clarity (Art. 6 PCT):*

Claims 70-76 define parameters of at least a portion of the nanoparticles or antigen-nanoparticle conjugates referred to. As the said portion is not defined the scope of said claims is rendered unclear.

What is claimed is:

1. A method of preparing antigen-nanoparticle conjugates, comprising:

providing a nanoparticle; and

conjugating a plurality of carbohydrate antigens to the nanoparticle, wherein the carbohydrate antigens include TF antigen, T_n antigen, Gb1 antigen, GM_1 antigen, GM_3 antigen, Lewis Y Antigen, or any combination thereof.

2. The method of claim 1, wherein a plurality of nanoparticles are provided, and a plurality of carbohydrate antigens are conjugated to at least a portion of the nanoparticles.

3. The method of claim 2, wherein a plurality of carbohydrate antigens are conjugated to each of the nanoparticles.

4. The method of claim 1, wherein the carbohydrate antigens comprise a linking group, and the linking group is conjugated to the nanoparticle.

5. The method of claim 4, wherein the linking group includes at least one sulfur atom, carboxylate group, amide group, carbamate group, carbonate group, thiocarbamate group, thiocarbonate group, thioether group, succinamide group, n-hydroxy succinamide group, or any combination thereof.

6. The method of claim 1, wherein conjugating includes self-assembly of the carbohydrate antigens on at least one surface of the nanoparticle.

7. The method of claim 1, wherein the nanoparticle comprises one or more nanoparticle linking groups, and the carbohydrate antigens are conjugated to the nanoparticle linking groups.

8. The method of claim 1, wherein one or more spacer groups link the carbohydrate antigens to the nanoparticle.

9. The method of claim 8, wherein the one or more spacer groups include PEG, a carbon chain, a carbon chain including sulfur, nitrogen or oxygen in the backbone, polymers including polyacrylamide, a peptide chain made up of all glycine or alanine units, or any combination thereof.

10. The method of claim 1, further comprising purifying the nanoparticles.

11. The method of claim 10, wherein said purifying includes filtration, centrifugation, electrophoresis, chromatography, crystallization, or any combination thereof.

12. An antigen-nanoparticle conjugate comprising a plurality of carbohydrate antigens conjugated to a nanoparticle, wherein the carbohydrate antigens include TF antigen, T_n antigen, Gb1 antigen, GM_1 antigen, GM_3 antigen, Lewis Y Antigen, or any combination thereof.

13. The antigen-nanoparticle conjugate of claim 12, wherein the plurality of carbohydrate antigens are identical to each other.

14. (Cancelled).

15. The antigen-nanoparticle conjugate of claim 12, wherein at least a portion of the carbohydrate antigens are displayed to the immune system in human carcinomas during tumor growth and progression.

16. The antigen-nanoparticle conjugate of claim 12, wherein the carbohydrate antigens are capable of inducing galectin-3 surface expression in endothelial cells.

17. The antigen-nanoparticle conjugate of claim 12, wherein the carbohydrate antigens include at least one different carbohydrate antigen in addition to TF-Antigen.

18. The antigen-nanoparticle conjugate of claim 12, wherein at least one of the plurality of tumor–associated carbohydrate antigens is conjugated to a spacer group, and the spacer group is conjugated to the nanoparticle.

19. The antigen-nanoparticle conjugate of claim of 18, wherein the spacer group includes PEG, a carbon chain, a carbon chain including sulfur, nitrogen or oxygen in the backbone, a polymer, a peptide, or any combination thereof.

20. The antigen-nanoparticle conjugate of claim 12, wherein one or more carbohydrate antigens are conjugated to one or more linking groups, and the linking groups are conjugated to the nanoparticle.

21. The antigen-nanoparticle conjugate of claim 20, wherein the linking groups include at least one sulfur atom, carboxylate group, amide group, carbamate group, carbonate group, thiocarbamate group, thiocarbonate group, thioether group, succinamide group, n-hydroxy succinamide group, or any combination thereof.

22. The antigen-nanoparticle conjugate of claim 12, wherein one or more carbohydrate antigens are conjugated to one or more spacer groups, the spacer groups are conjugated to one or more linking groups, and the one or more linking groups are conjugated to the nanoparticle.

23. The antigen-nanoparticle conjugate of claim 22, wherein the linking groups include at least one sulfur atom, carboxylate group, amide group, carbamate group, carbonate group, thiocarbamate group, thiocarbonate group, thioether group, succinamide group, n-hydroxy succinamide group, or any combination thereof.

24. The antigen-nanoparticle conjugate of claim 12, wherein each of the carbohydrate antigens are covalently linked to one or more sulfur atoms.

25. The antigen-nanoparticle conjugate of claim 12, wherein the carbohydrate antigens are each linked, individually, to one or more sulfur atoms.

26. The antigen-nanoparticle conjugate of claim 12, wherein each of the carbohydrate antigens include one or more amino acids.

Replacement Sheet

27. The antigen-nanoparticle conjugate of claim 24, wherein at least two sulfur atoms are covalently linked to each other.

28. The antigen-nanoparticle conjugate of claim 24, wherein at least one of the sulfur atoms is bonded to the nanoparticle.

29. The antigen-nanoparticle conjugate of claim 28, wherein the bonds between the sulfur atoms and the nanoparticle include covalent bonds, hydrogen bonds, ionic bonds, van der Waals bonds, or any combination thereof.

30. The antigen-nanoparticle conjugate of claim 12, wherein the carbohydrate antigens include a disaccharide.

31. The antigen-nanoparticle conjugate of claim 30, wherein the disaccharide comprises at least one amino sugar group.

32. The antigen-nanoparticle conjugate of claim 12, wherein at least one of the plurality of the carbohydrate antigens is a prognostic indicator for cancer, a marker of metastasized carcinoma cells, an adhesion molecule involved in metastasis, or any combination thereof.

33. The antigen-nanoparticle conjugate of claim 12, wherein the nanoparticle includes gold atoms, silver atoms, platinum atoms, rhodium atoms, palladium atoms, or any combination thereof.

34. The antigen-nanoparticle conjugate of claim 33, wherein the nanoparticle is derived from colloidal gold.

35. The antigen-nanoparticle conjugate of claim 12, wherein the antigen-nanoparticle conjugate has a molecular weight in the range of from about 1,000 Daltons to about 1 million Daltons.

Replacement Sheet

36. The antigen-nanoparticle conjugate of claim 12, wherein the number of carbohydrate antigens conjugated to the nanoparticle is in the range of from about 2 to about 1000.

37. The antigen-nanoparticle conjugate of claim 12, wherein the nanoparticle has from about 50 to about 10,000 atoms.

38. The antigen-nanoparticle conjugate of claim 12, wherein the nanoparticle has a dimension in the range of from about 0.5 nm to about 100 nm.

39. The antigen-nanoparticle conjugate of claim 38, wherein the nanoparticle has a dimension in the range of from about 1 nm to about 10 nm.

40. The antigen-nanoparticle conjugate of claim 38, wherein the nanoparticle has a spheroidal shape, and the diameter of the nanoparticle is in the range of from about 1 nm to about 10 nm.

41. The antigen-nanoparticle conjugate of claim 38, wherein the nanoparticle has a icosahedral, cubic, rhombic, hexagonal, or fullerene shape.

42. (Cancelled).

43. A method for inhibiting metastasis of carcinoma cells in a mammal, comprising:

administering to a mammal or cells thereof a therapeutically effective amount of antigen-nanoparticle conjugates comprising a plurality of carbohydrate antigens conjugated to a plurality of nanoparticles, wherein the carbohydrate antigens include TF antigen, T_n antigen, Gb1 antigen, GM_1 antigen, GM_3 antigen, Lewis Y Antigen, or any combination thereof.

44. The method of claim 43, wherein the mammal is a human.

45. The method of claim 44, wherein the human is diagnosed as having cancer.

46. The method of claim 45, wherein the cancer is breast cancer.

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47. The method of claim 46, wherein metastasis to lung cells is inhibited.

48. The method of claim 43, further comprising removing tumor cells from said mammal.

49. The method of claim 48, wherein the tumor cells are removed from said mammal prior to administering the therapeutically effective amount of the antigen-nanoparticle conjugates.

50. The method of claim 43, wherein the carbohydrate antigens are displayed to the immune system in human carcinomas during tumor growth and progression.

51. (Cancelled).

52. The method of claim 43, wherein the carbohydrate antigens are capable of inducing galectin-3 surface expression in endothelial cells.

53. The method of claim 43, wherein the carbohydrate antigens include TF-Antigen.

54. The method of claim 43, wherein the carbohydrate antigens are conjugated to an exterior surface of said nanoparticle.

55. The method of claim 43, wherein at least one of the tumor–associated carbohydrate antigens is conjugated, individually, to at least one spacer group, and the at least one spacer group is conjugated to the nanoparticle.

56. The method of claim of 55, wherein the spacer group includes PEG, a carbon chain, a carbon chain including sulfur, nitrogen or oxygen in the backbone, a polymer, a peptide, or any combination thereof.

57. The method of claim 43, wherein one or more carbohydrate antigens are conjugated to a linking group.

58. The method of claim 43, wherein one or more carbohydrate antigens are conjugated, individually to a spacer group, and one or more spacer groups conjugated to a linking group.

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59. The method of claim 58, wherein the linking group include at least one sulfur atom, carboxylate group, amide group, carbamate group, carbonate group, thiocarbamate group, thiocarbonate group, thioether group, succinamide group, n-hydroxy succinamide group, or any combination thereof.

60. The method of claim 43, wherein each of the carbohydrate antigens is covalently linked to one or more sulfur atoms.

61. The method of claim 60, wherein at least two of the sulfur atoms are bonded to each other.

62. The method of claim 60, wherein the carbohydrate antigens are each linked, individually, to one or more sulfur atoms.

63. The method of claim 60, wherein at least one of the sulfur atoms is bonded to at least one of the plurality of nanoparticles using $NaBH_4$.

64. The method of claim 63, wherein the bonds between the sulfur atoms and the nanoparticle are characterized as being covalent bonds, ionic bonds, hydrogen bonds, van der Waals bonds, or any combination thereof.

65. The method of claim 43, wherein the carbohydrate antigens comprises a disaccharide.

66. The method of claim 65, wherein the disaccharide comprises at least one amino sugar group.

67. The method of claim 43, wherein one of the carbohydrate antigens is a prognostic indicator, a marker of metastasized carcinoma cells, an adhesion molecule involved in metastasis, or any combination thereof.

68. The method of claim 43, wherein at least one of the plurality of the nanoparticles includes gold atoms, silver atoms, platinum atoms, rhodium atoms, palladium atoms, or any combination thereof.

69. The method of claim 68, wherein the nanoparticles include colloidal gold particles.

70. The method of claim 1, wherein the molecular weights of at least a portion of the antigen-nanoparticle conjugates is in the range of from about 1,000 Daltons to about 1 million Daltons.

71. The method of claim 70, wherein the molecular weights of at least a portion of the antigen-nanoparticle conjugates is in the range of from about 10,000 Daltons to about 500,000 Daltons.

72. The method of claim 43, wherein at least a portion of the antigen nanoparticle conjugates comprise, individually, from 2 to about 1000 carbohydrate antigens.

73. The method of claim 43, wherein at least a portion of the nanoparticles comprise from about 50 to about 10,000 atoms.

74. The method of claim 43, wherein at least a portion of the nanoparticles has a dimension in the range of from about 0.5 nm to about 100 nm.

75. The method of claim 74, wherein at least a portion of the nanoparticles has a dimension in the range of from about 1 nm to about 10 nm.

76. The method of claim 74, wherein at least a portion of the nanoparticles comprises a spheroid shape, and the dimension is the diameter of the nanoparticle.

77-80. (Cancelled).